

Variations in serum concentrations of penicillin after injections of aqueous procaine penicillin G with and without oral probenecid

C. E. CORNELIUS III,* A. L. SCHROETER,† A. LESTER,‡ AND J. E. MARTIN Jr.§

Venereal Disease Research Laboratory, Atlanta, Georgia, U.S.A.

Probenecid by inhibiting the renal excretion of penicillin raises its serum level (Boger, Beatty, Pitts, and Flippin, 1950; Burnell and Kirby, 1951; Meads, Knight, and Izlar, 1951; Frisk, Diding, and Wallmark, 1952), a pharmacological effect of which increasing use has been made in the treatment of gonorrhoea (Hilton, 1959; Jensen, Kvorning, and Nørredam, 1963; Holmes, Johnson, and Floyd, 1967; Holmes, Johnson, Stewart, and Kvale, 1968; Editorial *B.M.J.*, 1968a; Olsen and Lomholt, 1969; Keys, Halverson, and Clarke, 1969).

Willcox (1970), in a recent review, has drawn attention to the paucity of data on penicillin serum levels when the antibiotic is used, with or without probenecid, in the large doses now required to overcome partially resistant gonococci. Few patients have been studied and notable variations have been reported by different investigators and sometimes by the same investigator even on the same subjects. The purpose of this report is to provide data of serum levels attained with the dosage of aqueous procaine penicillin G currently recommended by the United States Public Health Service for the treatment of gonorrhoea in men (2.4 mega units) and women (4.8 mega units) with and without the addition of 2 g. probenecid.

Methods and materials

To obtain baseline data, a detailed study of the serum levels of penicillin was made on eight male patients with gonorrhoea who were treated at the Atlanta Federal Prison, Atlanta, Georgia, with 2.4 m.u. aqueous procaine penicillin G (APPG) without the addition of probenecid.

Received for publication April 15, 1971

Requests for reprints should be sent to Mr. J. E. Martin, Venereal Disease Research Laboratory, Venereal Disease Branch, State and Community Services Division, Center for Disease Control, Atlanta, Georgia 30333, U.S.A.

*Department of Dermatology, Hospital of University of Pennsylvania, Philadelphia, Pennsylvania 19104, U.S.A.

†Venereal Disease Branch, State and Community Services Division, Center for Disease Control, Atlanta, Georgia 30333 U.S.A.

‡Bacteriologist, Venereal Disease Research Laboratory

§Supervisory Research Microbiologist, Venereal Disease Research Laboratory

Blood specimens were drawn at 1, 2, 3, 4, 6, 9, 12, 18, 24, 36, 48, and 72 hours after treatment to obtain detailed information on penicillin levels. The determinations were done at the Center for Disease Control by the *Sarcina lutea* cup plate method (Grove and Randall, 1955). Medical histories were taken and physical examinations were performed on all patients. Efforts were made to rule out possible physiological abnormalities by carrying out estimations such as hematocrit, blood cell count, urine analysis, and blood urea nitrogen. All the patients were under 35 years of age.

The main investigation concerned 44 patients, including nine women, all of whom required antibiotic therapy. These patients were divided into four equal groups and treated with one of the following regimens: 2.4 or 4.8 m.u. APPG with and without the addition of a single 2 g. oral dose of probenecid. The probenecid was administered at the time of injection. The 4.8 m.u. dosage of penicillin was divided into two equal injections administered at two sites (lateral upper quadrant of each buttock). All the women and two of the men had gonorrhoea proven by culture. The remaining patients had pyoderma. 10 ml. blood for determination of penicillin levels was drawn before therapy and 3, 6, 24, and 48 hours after treatment. The serum was frozen in dry ice and sent to the Eli Lilly Company, Indianapolis, Indiana, for determination of serum levels, again using the *Sarcina lutea* cup method. Treatment of the nine women who had gonorrhoea was as follows: two received 2.4 m.u. APPG, two 4.8 m.u. APPG, and the remaining five 4.8 m.u. APPG plus 2 g. of probenecid. All of the ten male patients with gonorrhoea (two from the main group of 44 and the eight in the initial series) received 2.4 m.u. APPG. As a test of cure, cultures of genital secretions were repeated one week after treatment. In addition all patients were interviewed and examined for gastrointestinal and cutaneous reactions at the time of blood drawing and reculturing.

Results

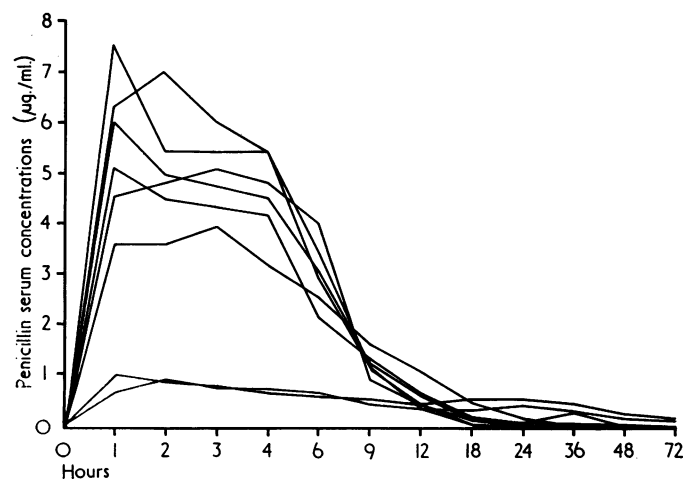
All patients with gonorrhoea had negative results to cultures one week after treatment. None of the patients in the study had any untoward reactions to the therapy.

Results of all the laboratory studies on the eight patients with gonorrhoea treated at the Atlanta

Federal Prison were within normal limits. The penicillin serum assays of these patients treated with 2.4 m.u. APPG without probenecid are presented in Table I. Approximately an 8-fold variation in peak serum penicillin levels is noted in the first 6 hours. This variation can be seen in the Figure, which shows that two of the eight patients failed to achieve a high peak level, *i.e.* greater than 1 $\mu\text{g./ml.}$, but had a low serum level persisting for an extended period. The area under the curve of the individual serum penicillin assays was computed in microgram hours ($\mu\text{g./hr.}$). The average $\mu\text{g./hr.}$ was 36 (range 25 to 41).

TABLE I *Penicillin serum levels ($\mu\text{g./ml.}$) attained after administration of 2.4 m.u. APPG (8 patients)*

Time (hrs)	Penicillin serum levels		
	Range	Average	Median
1	0.66-7.50	4.33	4.30
2	0.84-6.93	3.99	4.65
3	0.72-6.0	3.87	4.52
4	0.66-5.40	3.59	4.32
6	0.56-3.96	2.40	2.70
9	0.40-1.62	1.05	1.22
12	0.36-1.08	0.54	0.41
18	0.04-0.51	0.24	0.16
24	0 -0.51	0.16	0.09
36	0 -0.39	0.13	0.03
48	0 -0.25	0.06	0
72	0 -0.14	0.03	0



Tables II and III show the serum assays of the four groups of eleven patients treated with 2.4 or 4.8 m.u. APPG with or without 2 g. probenecid. The ranges of peak serum levels vary in magnitude more than those found in the study of the eight male patients treated with 2.4 m.u. alone (Table I). The ranges and averages tend to be higher among the patients who received probenecid.

TABLE II *Penicillin serum levels ($\mu\text{g./ml.}$) attained after administration of 2.4 m.u. APPG with and without the addition of a 2 g. oral dose of probenecid*

Dosage	Time (hrs)	Penicillin serum levels		
		Range	Average	Median
2.4 m.u. APPG	3	1.50-8.52	4.21	4.39
	6	1.70-6.18	4.32	4.58
	24	0.39-1.82	1.12	1.04
	48	0 -0.62	0.26	0.21
2.4 m.u. APPG plus 2g. probenecid	3	0.96-17.52	7.82	6.12
	6	1.43-12.00	7.23	6.51
	24	0 -2.14	0.49	0.24
	48	0 -0.37	0.21	0.05

Although there were fewer determinations, the general pattern observed in the Figure was true for the serum assays of patients receiving probenecid. The patients who gained an early high peak level had an earlier drop in serum levels, whereas those who failed to achieve high peaks continued to have low levels for an extended period. Calculation of the

FIGURE *Penicillin serum concentrations ($\mu\text{g./ml.}$) in eight male patients with gonorrhoea treated with a single intramuscular injection of 2.4 m.u. APPG*

TABLE III *Penicillin serum levels ($\mu\text{g./ml.}$) attained after administration of 4.8 m.u. APPG with and without the addition of a 2 g. oral dose of probenecid*

Dosage	Time (hrs)	Penicillin serum levels		
		Range	Average	Median
4.8 m.u. APPG	3	1.14-15.12	7.13	7.12
	6	0.58-13.96	5.67	6.48
	24	0.13-1.88	1.13	1.14
	48	0 -1.19	0.36	0.15
4.8 m.u. APPG plus 2g. probenecid	3	2.94-17.45	7.14	5.70
	6	2.10-41.75	14.48	12.42
	24	0.60-4.80	2.01	2.24
	48	0 -1.64	0.89	0.59

total $\mu\text{g./hr}$ of penicillin for the average peak level showed that patients receiving 2.4 m.u. APPG achieved an average of 85 $\mu\text{g./hr}$, those receiving 2.4 m.u. APPG plus probenecid, 112 $\mu\text{g./hr}$, those receiving 4.8 m.u. APPG, 147 $\mu\text{g./hr}$, and those receiving 4.8 m.u. APPG plus probenecid, 225 $\mu\text{g./hr}$. In other words, the patients who received the probenecid enjoyed an average of 31 and 56 per cent. greater total $\mu\text{g./hr}$, respectively.

Discussion

Frisk and others (1952) showed that probenecid significantly blocks the excretion of penicillin, thus enhancing peak blood levels. Other investigators have claimed 2- to 5-fold peak augmentation by probenecid, but the numbers of subjects were too small to show significant differences and the assay periods were short, *e.g.* 12 hours (Boger and others, 1950; Meads and others, 1951; Burnell and Kirby, 1951).

In the study of eight patients treated with 2.4 m.u. APPG alone (Table I), approximately an 8-fold variation was noted in the peak blood levels during the first 6 hours. This large biological variation is the factor that complicates penicillin studies, for large numbers of subjects are needed to compile data showing statistically significant differences in peak serum penicillin levels obtained by various regimens. The data presented in Tables II and III show that in general the range and averages of serum levels achieved with 2.4 and 4.8 m.u. APPG tend to be higher among those patients receiving the probenecid, but an 8-fold biological variation could easily mask the potentiating effect of probenecid. It is, therefore, impossible to determine the exact potentiating effect of probenecid from these data.

Even when the data from Tables II and III were corrected for the body weight of the patient, the serum assay variation was between 5- and 8-fold.

The large variation in individual serum peak assays noted in the first 4 hours after treatment suggests that the source of variation lies in the release of penicillin from the site of injection rather than catabolism or excretion (Table I). This hypothesis is based upon exclusion of differences in the rates of metabolism and excretion of penicillin in the eight patients: the majority of an intramuscular dose of penicillin is eliminated in the urine, and differences in rates of tubular excretion are unlikely to account for large differences in peak serum assays. The maximum tubular excretory capacity of the normal male for intravenously administered crystalline penicillin is approximately 3 m.u. per hour (Bryner, Clark, Randall, and Rantz, 1948). It would seem that the rate of release of penicillin from the intramuscular depot is the major determinant of serum levels when dealing with doses of this magnitude over a 4-hour period, and this is supported by the fact that the total $\mu\text{g./hr}$ of penicillin in the blood calculated for each patient showed comparatively little variation (25 to 41 $\mu\text{g./hr}$). Each patient therefore enjoyed nearly the same total $\mu\text{g./hr}$ despite wide variations in peak levels. One of the main reasons for treatment failure may indeed be related to the factors that control release of penicillin from the site of injection.

Nevertheless, in recent treatment studies of gonorrhoea, probenecid has been shown to raise the percentage cure rate. Holmes and others (1967), in a study of groups of approximately sixty patients, found that probenecid in a dosage of 1 g. 1 hour before, and 500 mg. 6, 12, and 18 hours after treatment raised the cure rate with a 2.4 m.u. dose of penicillin from 71 to 98 per cent. In another study of 1,263 patients, using the same form of treatment, a 28 per cent. increase in cure rate was noted (Holmes and others, 1968). F. R. Curtis, in a study of three groups each of over 600 male patients, found that the addition of a single oral dose of 2 g. probenecid reduced treatment failure rates from 6.2 to 2.9 per cent. when using 0.6 m.u. APPG, from 2.5 to 1.6 per cent. when using 1.2 m.u. APPG, and from 1.4 to 0.3 per cent. when using 2.4 m.u. APPG (Leader, *B.M.J.*, 1968a).

Although multiple dose probenecid therapy would probably provide the highest blood levels of greatest durations, the effect of a single 2 g. dose administered concurrently with the penicillin was studied in our work. In an outpatient clinic where there is a lack of continuous patient control, divided dosage schedules are unreliable and it is doubtful if they are necessary. According to Boger and others (1950) and Meads

and others (1951), the excretion of penicillin is blocked in proportion to the increasing dosage of probenecid from 0.5 to 4 g.: a single 1 g. dose of probenecid persists for as long as 24 hours and a 2 g. dose would probably be detectable after a longer interval. A 2 g. dose is the maximum for a 24-hour period approved by the U.S. Food and Drug Administration. There appears to be no need to give probenecid before the administration of penicillin because probenecid is rapidly absorbed from the gastrointestinal tract (Boger and others, 1950), providing an effective block to the excretion of penicillin which persists on an average from 3 to 4 hours (Table I).

The safety of probenecid has been well established. A single daily 2 g. dose has been shown to be well tolerated by the average patient for several years (Boger and Strickland, 1955; Bartels, 1960; Leader, *B.M.J.*, 1968b). Gastrointestinal intolerance and skin reactions are the principal though infrequent forms of intolerance, and other toxic effects are rare indeed: Ferris, Morgan, and Levitin (1961) and Sokol, Bashner, and Okun (1967) have reported nephrotic syndromes due to probenecid. The probability of causing a gouty attack or renal damage with a single dose is extremely small.

Conclusions

Probenecid, as an adjuvant to penicillin, has been used with success to improve the cure rates of gonorrhoea. Although there is no statistically significant evidence showing the exact degree of augmentation that probenecid has upon the serum levels of penicillin, the data show that in general probenecid in a single dose does act effectively to raise the serum penicillin levels.

Physicians should be aware that wide variations in serum concentration follow the administration of procaine penicillin by intramuscular injection with or without the use of probenecid, and that these variations are likely to have an effect upon cure rates. The levels of penicillin in serum achieved with 4.8 m.u. APPG plus 2 g. probenecid should be sufficient to cure the great majority of patients with gonorrhoea, bearing in mind that, in a recent study in the United States (Martin, Lester, Kellogg, and Thayer, 1969), strains of gonococci isolated in cases of treatment failure showed minimum inhibitory concentrations with penicillin ranging from 0.003 to 1.32 $\mu\text{g./ml.}$ Nevertheless one notes with caution the recent isolation of a strain with a MIC of 3.5 $\mu\text{g./ml.}$ (unpublished data—Venereal Disease Research Laboratory, Center for Disease Control, Atlanta, Georgia).

Summary

Eight male patients were treated with 2.4 mega units aqueous procaine penicillin G (APPG) and penicillin serum assays were performed on blood drawn at 1, 2, 3, 4, 6, 9, 12, 18, 24, 36, 48, and 72 hours after therapy. Approximately an 8-fold variation in peak serum penicillin levels was noted. In addition four groups of eleven patients were treated with 2.4 and 4.8 m.u. APPG, with and without the addition of a 2 g. oral dose of probenecid. Penicillin serum assays were performed on blood drawn at 0, 3, 6, 24, and 48 hours. Although the range and averages of peak penicillin serum concentrations were higher in those patients receiving probenecid, biological variation tended to mask the effect of probenecid. This variation may be due to the factors governing the release of penicillin from the injection site and may subsequently affect cure rates.

We are indebted to the Eli Lilly Company, Indianapolis, Indiana, for their assistance in the determination of penicillin serum levels and to the Merck Sharp and Dohme Company for the provision of probenecid (Benemid).

We should also like to thank Dr. James F. Hackney, District Director of Public Health, Fulton County Health Department, Atlanta, Georgia, for allowing us to carry out the clinical portion of the study at the Key Road Prison.

References

- BARTELS, E. C. (1960) *Med. Clin. N. Amer.*, **44**, 447
- BOGER, W. P., BEATTY, J. O., PITTS, F. W., and FLIPPIN, H. F. (1950) *Ann. intern. Med.*, **33**, 18
- and STRICKLAND, S. C. (1955) *Arch. intern. Med.*, **95**, 83
- Brit. med. J.* (1968a) Leading article, **3**, 391
- (1968b) Leading article, **3**, 569
- BRYNER, S., CLARK, W. H., RANDALL, E., and RANTZ, L. A. (1948) *Amer. J. Med.*, **5**, 202
- BURNELL, J. M., and KIRBY, W. M. (1951), *J. clin. Invest.*, **30**, 697
- FERRIS, T. F., MORGAN, W. S., and LEVITIN, H. (1961) *New Engl. J. Med.*, **265**, 381
- FRISK, A. R., DIDING, N., and WALLMARK, G. (1952) *Scand. J. clin. Lab. Invest.*, **4**, 83
- GROVE, D. C., and RANDALL, W. A. (1955) 'Assay Methods of Antibiotics, A Laboratory Manual', pp. 7-16. Medical Encyclopedia Inc., New York
- HILTON, A. L. (1959) *Brit. J. vener. Dis.*, **35**, 249
- HOLMES, K. K., JOHNSON, D. W., and FLOYD, T. M. (1967) *J. Amer. med. Ass.*, **202**, 461
- HOLMES, K. K., JOHNSON, D. W., STEWART, S., and KVALE, P. A. (1968), *Milit. Med.*, **133**, 642
- JENSEN, P. E., KVORNING, S. A., and NØRREDAM, K. (1963) *Brit. J. vener. Dis.*, **39**, 238
- KEYS, T. F., HALVERSTON, C. W., and CLARKE, E. J., Jr. (1969) *J. Amer. med. Ass.*, **210**, 857
- MARTIN, J. E., LESTER, A., KELLOGG, D. S., and THAYER, J. D. (1969) *Appl. Microbiol.*, **18**, 21

- MEADS, M., KNIGHT, V. H., and IZLAR, H. L., Jr. (1951) *S. med. J. (Bgham, Ala.)*, **44**, 297
- OLSEN, G. A., and LOMHOLT, G. (1969) *Brit. J. vener. Dis.*, **45**, 144
- SOKOL, A., BASHNER, M. H., and OKUN, R. (1967) *J. Amer. med. Ass.*, **199**, 43
- WILLCOX, R. R. (1970) *Brit. J. vener. Dis.*, **46**, 217.

Variations dans les concentrations sériques de pénicilline après injections de pénicilline-procaïne G aqueuse, avec ou sans probenecid oral

SOMMAIRE

Huit hommes furent traités avec 2,4 méga unités de pénicilline procaïne aqueuse G (APPG) et des études

sur les taux sériques de pénicilline furent entreprises après 1, 2, 3, 4, 6, 9, 12, 18, 24, 36, 48 et 72 heures après ce traitement. Approximativement, des variations allant jusqu'à 8 fois plus furent notées dans le maximum du taux sérique de pénicilline. En outre, 4 groupes de 11 malades furent traités avec 2,4 et 4,8 méga unités d'APPG avec ou sans l'addition d'une dose orale de 2g. de probénécid. Des dosages de pénicilline dans le sérum furent faits après 0, 3, 6, 24, et 48 heures. Bien que le niveau et les moyennes du maximum de pénicilline dans le sérum fussent supérieurs chez les malades recevant du probénécid, la variation biologique eut une tendance à masquer les effets du probénécid. Cette variation peut être due à des facteurs dont dépendent la libération de la pénicilline du point de l'injection et peut, en conséquence, affecter l'effet thérapeutique.